complex biological problems that simply can not be addressed through traditional biochemical approaches. The primary advantages of our approaches are that we can actually see what proteins are bound to DNA, where they are bound, how they move, and how they influence other components of the system - all in real-time, at the level of a single reaction. Our research program is focused on studying the regulation and activity of proteins that are involved in repairing damaged chromosomes. We are particularly interested in determining the physical basis for the mechanisms that proteins use to survey DNA molecules for damage and initiate repair processes, and how these initial steps are coordinated with downstream events that lead to completion of repair. As part of our work, we are also actively pursuing the development of novel experimental tools that can be used to facilitate the study of single biochemical reactions. In particular, we are applying techniques derived from nanotechnology to our biological research, and using nano- and micro-scale engineering to facilitate the development of new, robust experimental platforms that enable ''high throughput'' single molecule imaging.

962-Pos

Watching Conformational and Photo-Dynamics of Single Fluorescent Proteins in Solution

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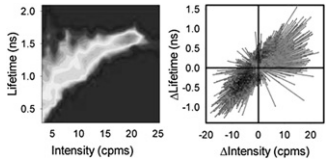
Observation of dynamics of single biomolecules over a prolonged time period without significantly altering the biomolecule via immobilization is a difficult challenge. This result is achieved with the Anti-Brownian ELectrokinetic (ABEL) Trap, which allows extended investigation of solution-phase biomolecules without immobilization via real-time electrokinetic feedback. We apply the ABEL trap to study an important photosynthetic antenna protein, Allophycocyanin (APC). Single molecules of solutionphase APC can often be studied for more than one second. We observe a complex relationship between fluorescence intensity and lifetime that cannot be explained by simple static kinetic models. Light-induced conformational changes are shown to occur. Further, evidence is obtained for fluctuations in the spontaneous emission lifetime, which is typically assumed to be constant. Our observations provide a new window into the dynamics of fluorescent proteins and are relevant for interpretation of in vivo singlemolecule imaging experiments, bacterial photosynthetic regulation, and biomaterials for solar energy harvesting.



963-Pos

What Can We Learn From Single-Molecule Diffusion

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There is increasing interest in a detailed understanding of the structure and dynamics of the cellular plasma membrane, primarily based on recognizing its essential role for controlling cellular signaling processes. Various pictures emerged, which ascribe the plasma membrane a high degree of organization at very short length scales of tens of nanometers. We employed single molecule fluorescence microscopy to study diffusion of CD59, a GPI-anchored protein, in the plasma membrane of living T24 cells at sub-wavelength resolution, both on the cell body and on tunneling nanotubules connecting cells. By separating longitudinal and transversal mobility, we found isotropic diffusion behavior on the surface of tunneling nanotubules, rendering direct influences of the membrane skeleton unlikely.

In both studies we analyzed the mean square displacement as a function of the time-lag and the distribution of displacement steps. However, a closed analytical theory for these analysis is only available for the simplest models. To address a suspected diffusion process we reasoned that a full analytical description may not be required; it may well be sufficient to compare the experimental data with Monte Carlo simulations of the process. We demonstrated the working principle for the analysis of free diffusion, hop diffusion and transient binding of the tracer molecule to slowly moving receptors.

In the recent years increasing evidence was reported for an inherent heterogeneity of cell populations. Our reasoning was that mobility probes nanometer-sized properties of the moving protein and its local environment. Automated and tailored data analysis routines allowed for the analysis of the required large data sets: ~200.000 trajectories obtained on ~350 cells were analyzed in total. We found up to five-fold higher variability of the diffusion constant between cells compared to the uncertainty for the determination of the diffusion constant on a single cell.

964-Pos

Electrostatic Switching of Polysaccharide Conformation Probed at the Single Molecule Level

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Polysaccharides play a key mechanical role in maintaining cell integrity and in cell-cell recognition. Single molecule AFM stretching measurements have revealed that upon loading, the backbone of polysaccharide molecules change their conformation and these conformational changes depend on the linkages between the sugar rings. It has been proposed that these force-induced conformational transitions may play an important role in biological systems. However a mechanism to switch on/off these conformational transitions and control the nanomechanical properties of carbohydrates has not yet been shown.

Here we demonstrate an electrostatic switch that can be used to toggle the force dependent conformational transition in acidic polysaccharides. Using single molecule AFM force spectroscopy we show that the tension dependent conformation of the polysaccharide molecules can be controlled by varying the backbone charge density and solution electrostatics.

965-Pos

An Optical Conveyor for Molecules

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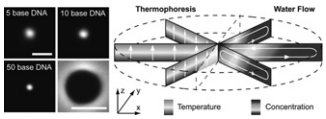
We optically trap molecules in free solution, which allows to accumulate 5-base DNA to a hundredfold excess within seconds [1]. The concentration of the trapped DNA scales exponentially with length, reaching trapping potential depths of 14kT for 50 bases. This novel way to trap molecules could be used to enhance diffusion-limited surface reactions, redirect cellular signaling, observe individual biomolecules over a prolonged time or separate small molecules in solution by their diffusion constant.

The mechanism is based on the microscale analog of a conveyor belt: a bidirectional flow, driven optically by the recently shown thermo-viscous fluid pump [2,3], is combined with a perpendicular thermophoretic molecule drift. Arranged in a toroidal geometry, no microfluidics, electrodes or surface modifications are required. As a result, the trap can be dynamically relocated.

[1] Weinert and Braun, Nano Letters, accepted
[2] Weinert, Kraus, Franosch and Braun, PRL 100, 164501 (2008)
[3] Weinert and Braun, JAP 104, 104701 (2008)



966-Pos

Recovering Absolute Fret Efficiency from Single Molecules: Comparing Methods of Gamma Correction

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Fluorescence resonance energy transfer is widely thought of as a ''spectroscopic ruler.'' Because biological processes and cellular assemblies occur on the nanometer scale, FRET is a popular tool for structural biology. In contrast to ensemble solution FRET measurements which record the entire emission spectrum, microscopy- based FRET experiments separate donor and acceptor intensity by passing the emission through a series of optical elements. Observed FRET efficiency, determined from the uncorrected donor and acceptor intensities, has been called a relative proximity ratio, which is internally consistent *only* if the photophysical properties and instrument remain unchanged. However, it is desirable to measure absolute distances using FRET, which requires that FRET efficiency be corrected for both instrument response and fluorophore properties. Thus, ''gamma'' correction adjusts for differences between the donor and acceptor dyes in their probability of photon emission upon excitation and the probability that emitted photons will be detected. Methods of gamma correction vary depending on the single molecule methodology. To test different methods for correcting FRET efficiency, we recorded smFRET distributions for protein and DNA on different instruments and with different filter sets which altered the observed FRET efficiency. Knowledge of filter set transmission allows for comparison of results between groups using different instruments. Applying empirically-derived corrections for instrument response and quantum yield was only slightly better than corrections based solely on filter set transmission data. We found that gamma correction based on single molecule photobleaching was the most effective particularly when gamma was determined for each sample or even each molecule. Variations in focus of the two colors and sub-pixel errors